Evaluating of 8-Hydroxy-Deoxyguanosine Interleukin-6, Interleukin-7 In Acute Kidney Injury Iraqi Males

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Abstract

Background: Acute kidney injury (previously known as acute renal failure) is a sudden loss in kidney function. There are a number of triggers, including blood and fluid loss from accident, dehydration, burns, or surgery, side effects from drugs, cardiovascular disease, infection, liver failure or severe allergic reaction.

Objective: The aim of the present study was to assess five important factors in patients with AKI (the amount of 8-OHDG, IL-6, IL-7, Blood Pressure, and Pulses). These factors were surveyed in AKI patients to compare with healthy controls and find out the correlation between AKI and serum 8-OHDG, IL-6, IL-7, Blood Pressure, and Pulses levels

Materials & Methods: The study was conducted prospectively between the dates June 2012 and April 2013. Population of the study consists of 41 patients diagnosed with acute kidney injury (AKI) and a control group of 41 healthy people. 8-OHDG, interleukin-6, interleukin-7 and blood pressure levels were evaluated in all subjects.

Results: Serum levels of inflammation markers, IL-6 and IL-7 were significantly increased in

AKI group versus control group. The percent of DNA damage of peripheral blood mononuclear cells was higher in AKI patients (23.24±10.84 ng/ml) compared to healthy controls (8.30±3.86 ng/ml) ($p<0.001$). Pearson correlation analysis showed a significant positive correlation of DNA damage with IL-6 and IL-7, but not with Sys.BP, Dia.BP, pulses and age.

Conclusion: the results of the present study suggest that 8-OHdG level in Acute kidney injury may be a useful marker of DNA damage also blood 8-OHdG had significantly positive correlation with IL-6 and IL-7. Oxidative stress may serve as a risk factor for the presence of Acute kidney injury in Iraqi men.

Key words: ● Acute kidney injury ● DNA Damage ● interleukin-6 ● interleukin-7 ●8-OHDG

Introduction

The kidneys are a vital organ with multiple important roles in maintaining organismal homeostasis. During the excretion of wastes and the reabsorption of water and nutrients, the kidneys are especially vulnerable to the effects of toxic compounds including drugs and metabolites. Impaired kidney function can be the result of either acute kidney injury (AKI) or chronic kidney disease (CKD). AKI can be caused by trauma, sepsis, or drug toxicity, while CKD may be a complication of diabetes mellitus, severe hypertension, autoimmune diseases or other chronic conditions. Assessment of kidney function has historically relied on measurements of blood pressure, serum creatinine, blood urea nitrogen (BUN), protein to creatinine ratio in urine, and

urine sediment, as well as changes in glomerular filtration rate (GFR).

These assessments are not very sensitive, may be delayed in AKI, and may not detect all types of chronic kidney damage. Furthermore, in experimental animals or in humans, substantial kidney damage may occur without a measurable change in GFR.(1)

Renal compromise can be categorized based on the anatomic location of the cause as prerenal (i.e. related to decreased renal perfusion and/or reduced glomerular blood flow), intrinsic (i.e., caused by a specific disease or injury to the kidneys, such as injury from nephrotoxic drugs or other substances), and postrenal (i.e. related to obstruction of the urinary tract). Patients with AKI that has a prerenal or a postrenal cause generally have a better prognosis than patients with ARF that involves intrinsic renal disease such as acute tubular necrosis (ATN) or interstitial nephritis.(2)

Oxidative stress is a factor in a spate of metabolic disturbances occurring in the course of AKI.(3) One of the methods to measure to assess oxidative stress is to measure the serum level of 8-hydroxy-2.-deoxyguanosine (8-OHdG) which is formed deoxyguanosine during DNA oxidation.(4)

We used 8-hydroxy-deoxyguanosine (8-OHDG or 8-OHOG) as a marker for ROS accumulation.(5) 8-OHDG is one of the major products formed upon oxidative damage of DNA in various pathological conditions.(6) 8-OHdG accumulation has been observed in circulating leukocytes and in various cell types in atherosclerotic lesions of human and animal models.(7)

The release of pro-inflammatory cytokines in AKI patients is often associated with increased production of reactive oxygen species (ROS), either as a component of the immune response or as a consequence of increased metabolism.(8) ROS, in turn, may inhibit erythropoiesis. Inflammation can also interfere with nutritional status, which in turn may induce anemia.(9) Patients with chronic renal failure (CRF) undergoing hemodialysis (HD) are exposed to persistent inflammatory state, as shown by elevated interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) plasma concentrations.

Erythropoietin resistance is known to be strongly associated with chronic inflammation.

Many studies observed a CD4+ lymphopenia associated with increased IL-7 serum levels, an activation stage of T-cells and an enhanced ability of these cells to produce Th1 related cytokines (IL-2, INF-γ and TNF-α) after short term in vitro stimulation. This increased capacity of T-cells to produce Th1 cytokines could justify, at least in part, the anaemia found in HD patients.(10) Other studies demonstrate that IL-7 stimulates the proliferation of cells derived from patients with chronic lymphocytic leukemia and the Sezary syndrome of cutaneous T cell lymphoma , the observation that IL-7 induced an increase in DNA synthesis in acute myelogenous leukemia cells suggests that the pleiotropic effect of this cytokine is not restricted to cells from the lymphoid lineage.(11)

High blood pressure is one of the leading causes of kidney failure. Hypertension may damage the blood vessels in the kidney and effect the secretion of waste product. Waste may secrete in extra cellular fluid and further rise the blood pressure eventually leading to ESRD.G-protein coupled and Ca2+ dependent kinases are responsible for the control of blood pressure. Mutations may cause changes in receptors, which in turn raise blood pressure.(12)

Material and Methods

The study was approved by a local ethics Committee and the subjects participating in the study
gave informed consent to study procedures.

The study group consisted of 41 hemodialysis patients due to AKI. All patients were
hemodialyzed with a carbohydrate solution. The weekly duration of dialysis was 12 h in 3-4-h
sessions, performed with a polysulfone dialyzer. No patient was receiving any permanent
pharmacological treatment known to influence lipid balance for 6 months before the onset of the
study or antioxidant vitamins during the study course. Smoking in anamnesis was an exclusion
criterion from the study. A control group consisted of 41 healthy persons, with no clinical symptoms

of any disease and with the markers of renal function in the norm.

All blood samples were collected from the ulnar vein, in the morning before a dialysis session. All subjects underwent full history taking and clinical examination including measuring blood pressure and pulses.

The analysis of the following markers in the serum was performed: 8-OHdG, IL-6 and IL-7.

Serum 8-OHdG was measured using an ELISA Kit, Cayman Chemical, MI, USA.(13) Serum IL-6 was determined by enzyme linked immunosorbent assay method using AviBion Human IL-6 ELISA kit, Ani Biotech Oy, Orgenium Laboratories Business Unit, FINLAND.and IL-7 also determined by enzyme linked immunosorbent assay method using kit manufactured by (Ray biotech. Company, USA).

Statistical Analysis

Descriptive analysis was performed. Categorical data are presented as a frequency table, and quantitative data were analyzed using the Statistical Package for Social Sciences (SPSS version 20, Sydney, NSW, Australia) and Microsoft Excel (Office2007, Microsoft). All values are expressed as mean ±S.D. Statistical analysis was performed using ANOVA. Pearson'scorrelation was performed to illustrate the correlation between 8-OhdGand IL-6, IL-7, Sys.BP, Dia.BP and Pulses, and Student t-test used to compare quantitative variables. For all statistical analyses, $P<0.05$ was considered statistically significant.

Results

Table 1 shows the baseline demographic characteristics of controls and AKI cases. Both controls and cases were well-matched with respect to age (49.69±15.63 vs 52.56±16.16 years).

There was a significant increase in serum 8-OHdG in AKI patients (23.24±10.84 ng/ml) compared with normal subjects (8.30±3.86 ng/ml),($P<0.0001$[HS]). Serum 8-OHdG was significantly and positively correlated with IL-6 and IL-7 (r= 0.686 and 0.636 respectively),(table 2). In contrast, Sys.BP, Dia.BP and pulses for AKI patients were not associated with serum 8-OhdG (table2).AKI patients also had a significantly higher IL-6 and IL-7 compared with normal subjects (7.61±3.20 vs 7.61±3.20 pg/ml, $P<0.0001$[HS) and (25.76±10.82 vs 10.18±3.92pg/ml, $P<0.0001$[HS) (table1 and figure 1). Sys.BP, Dia.BP and pulses were significantly higher in AKI patients compared with normal subjects(130.67±13.83 vs 119.13±7.53 mmHg, $P<0.0001$[HS), (81.27±8.36 vs 73.00±3.97 mmHg, $P<0.0001$[HS) and (79.16±7.72 vs 72.58±7.09 MIN-1, $P<0.0001$[HS) respectively (table1 and figure 1).

Discussion

Oxidative stress is defined as the imbalance between the formation of reactive oxygen species and antioxidants. Acute kidney injury is associated with oxidative stress, the mechanism of which is unknown. Biomarkers which are specific for oxidative damage of DNA include products of DNA fragmentation and oxidised bases such as 8-hydroxy-2-deoxyguanosine (8-OHdG).

In present study, the level of 8-OHdG was 2.8-fold greater in AKI patients compared with that healthy subjects, which may be taken as evidence of intensive oxidative stress in these patients, our results agreed with different studies (14,15) that demonstrated increased serum 8-OHdG levels AKI subjects.

A dialysis treatment is more or less biocompatible, depending of filter, flow and dialysis fluids used. However, even in the best cases, the blood is still re-circulated outside the body, which activates the complement system, coagulation and leukocytes mechanically by the dialysis filter, through exposure to air or by microbiologic exposure. (16)

In this study, we confirmed that serum IL-6, IL-7 and BP in AKI patients were significantly higher than in general subjects. These patients have increased inflammatory markers as a result of the uraemic condition before dialysis (pre-dialysis).(17)

Attention now is being focused on the increased oxygen consumtion per nephron as a consistent tubule adaptation that occur with nephron loss. The present study confirms the altered status of 8-

OHDG associated with high levels of IL-6 and IL-7. Our fiindings indicate that this situation exists before dialysis. In acute kidney injury, very highly significant increase in level of 8-OHDG before first hemodialysis, is due to increase in oxidative stress by production of free radicals and reactive oxygen species. Once free radicals attacks kidney there will be progressive development of disease, which lead to increase in the level of DNA damage. 8-OHdG is known to be a sensitive marker of oxidative DNA damage and of total systemic oxidative stress in vivo. Interestingly, 8-OHDG appears to play a role in tissue cell injury via the induction of apoptotic cell death , previous studies have shown that 8-OHdG is one of the commonly used markers for evaluation of oxidative DNA damage. (18)

An elevation of 8-OHdG indicates an increase in the degree of oxidative stress affecting tissue function and integrity and therefore provides useful information on oxidative stress and disease progression. (19)

Tissue deposition of immune complexes also can induce an acute inflammatory response resulting in tissue injury. Neutrophils get attracted to the site of immune complex deposition. Following activation, neutrophils undergo a 'respiratory burst' resulting in excessive production of oxygen free radicals, (20) before HD might be due to induction of nitric oxide synthase (NOS) in macrophages and in intrinsic glomerular cells which is mediated by the interactions of cytokines such as IL-6 and IL-7. Inducible nitric oxide synthase (iNOS)- generated NO; via peroxynitrite mediated cytotoxicity, might be able to play a major role in AKI.

The initiation of the inflammatory process in AKI can be associated with an elevation of serum anti-inflammatory cytokines, including IL-7 and some pro-inflammatory cytokines such as IL-6 and TNF-α. IL-6 is reported to play a central role in the pathophysiology of the adverse effects of inflammation in AKI patients. Increased activation of inflammatory cytokines, such as IL-6, may cause muscle breakdown and hypoalbuminemia and may be involved in atherogenesis. (21)

Markedly elevated circulating IL-6 levels are found in AKI patients , which may be due to impaired removal of cytokines, and increased synthesis due to various infectious processes, co-morbid conditions such as coronary heart disease, chronic heart failure, increased body fat mass, as well as other as yet unknown factors. (22,23)

Patients with chronic renal failure display higher inflammation levels and inflammatory factors in their blood compared to general population. Inflammatory factors increase vascular endothelial damage and thrombosis. Inflammation causes progression of kidney damage, as well as acceleration of decreased kidney function. In addition, higher levels of inflammation play a part in chronic rejection of kidney transplantation. (24)

In AKI patients, high levels of 8-OHDG seem to affect the generation of pro-inflammatory cytokines

[interleukin-6 (IL-6), and interleukin-6 (IL-7)]. In fact, high levels of those cytokines may induce muscle mass loss, reducing albumin synthesis, inhibiting appetite, and contributing to the development of malnutrition. (25) In addition, the association of

inflammation and oxidative stress has been reported in patients with CKD. Oxidative stress occurs in inflammation sites, during small injuries, and as part of the reaction to invasive microorganisms, that reaction causes the production of several reactive oxygen species (ROS), generating modified macromolecules, which then could be involved in the atherogenic process. (26)

We have provided data on the AKI risk associated with high blood pressure separately for middle-aged and elderly subjects.Men with high- blood pressure at base-line examination had a higher incidence of AKI disease on follow-up than those with optimal blood pressure. Although our results demonstrate that high-normalblood pressure is a marker of an elevated risk of AKI disease, it is uncertain whether the increased risk is attributable solely to subjects' bloodpressure levels.

In conclusion, increased serum 8OHDG predicts an increased risk for AKI patients, and increased oxidative stress may partially account for the risk for AKI associated with inflamanation. BP also predict the development of AKI,but , it is not evident that they contribute to the relationship

between IL6, IL-7 and AKI in these patients. Therapies that reduce intraoperative oxidative stress might reduce the incidence, severity, and associated morbidity of AKI patients

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Table 1: Comparison of demographics and biochemical profiles of controls and AKI cases

Parameters		Mean ± S. D	cohen's d	t	p-value
AGE YEARS	cases	52.56±16.16	0.244	-1.101	0.274
	controls	49.69±15.63			
IL-6 pg/ml	cases	7.61±3.20	1.712	-6.983	0.000
	controls	3.33±1.51			
IL-7 pg/ml	cases	37.42±10.82	0.168	0.850	0.000
	controls	15.95±3.92			
8-OHDG ng/ml	cases	23.24±10.84	1.837	-7.383	0.000
	controls	8.30±3.86			
SYS.PRESURE mmHg	cases	130.67±13.83	1.036	-4.269	0.000
	controls	119.13±7.53			
DIA.PRESURE mmHg	cases	81.27±8.36	1.264	-5.160	0.000
	controls	73.00±3.97			
PULSES 1/ MIN	cases	79.16±7.72	0.887	-3.856	0.000
	controls	72.58±7.09			

* Correlation is significant at the 0.0\ level (2-tailed).

Table 2: Correlation of 8-OHDG with IL-6, IL-7, Sys.BP, Dia.BP and pulses

Parameters	8-OHDG ng/ml
IL-6 pg/ml	0.686*
IL-7 pg/ml	0.636*
8-OHDG ng/ml	1
SYS.PRESURE mmHg	-0.095
DIA.PRESURE mmHg	0.086
PULSES 1/ MIN	0.199

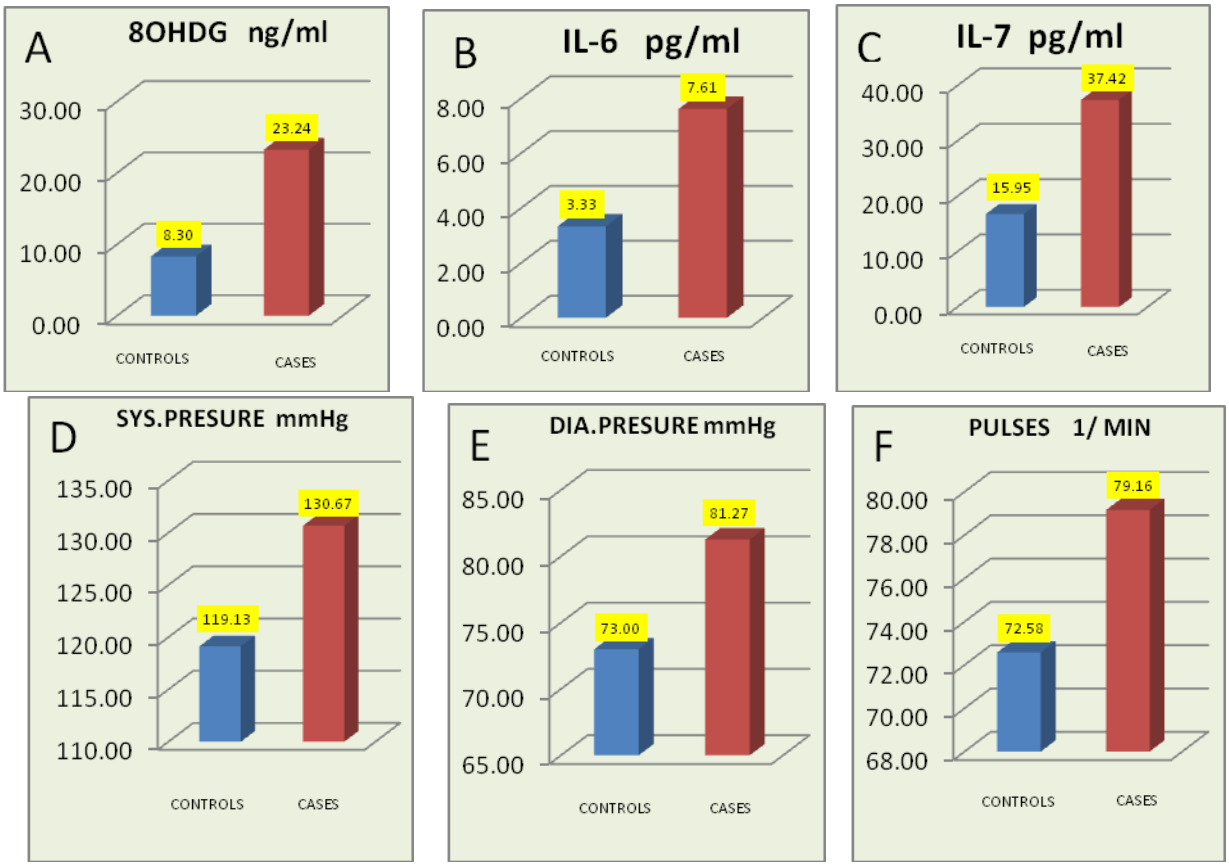

Fig. 1:The Serum 8-OHDG, IL-6 and IL-7, Sys.BP, Dia.BP, Pulses levels in AKI males compared with control samples.